characterized as an RTX leukotoxin from a bacterium selected from the group consisting of *Pasteurella haemolytica*, *E. coli* and *Actinobacillus pleuropneumoniae.* Additionally, dependent claims 44 and 45 have been added and recite that the leukotoxin polypeptide is a "*Pasteurella haemolytica* leukotoxin polypeptide."

Support for these amendments can be found throughout the specification at, *inter alia*, page 8, lines 24-32; page 10, lines 12-15; and page 11, lines 1-5. Accordingly, no new matter has been added to the application by way of these amendments.

A copy of the elected claims, incorporating the amendments made herein, is provided for the Examiner's convenience.

Formal Matters:

The Office requested that applicant comply with the Sequence Rules under 37 CFR §1.821-25 and provide a Sequence Listing and appropriate amendments to the application. However, applicants provided a Sequence Listing with the Preliminary Amendment that accompanied the filing of the application and amended the specification and claims as required. Applicants therefore request clarification.

The Double Patenting Rejections:

Claims 37, 40 and 41 were rejected under the judicially created doctrine of obviousness-type double patenting over claims 1, 2, 5, 6, and 9 of U.S. Patent No. 5,422,110, claims 1-23 of U.S. Patent No. 5,837,268 and claims 1-8 and 19-22 of U.S. Patent No. 5,723,129. Applicants will consider filing a Terminal Disclaimer upon the indication of allowable subject matter in the present application.

Rejections Under 35 USC §112, First Paragraph:

The Office objected to the specification and rejected claims 37, 40 and 41, under 35 USC §112, first paragraph. In particular, the Office argues that the specification

"makes broad reference to the preparation of a chimeric protein comprising any antigen and the leukotoxin, wherein certain cytokines have been exemplified as antigens at page 4." Office Action, page 5. The Office further asserts that the "term 'antigen' encompasses many different and diverse proteins" and that "enablement for three antigens (i.e., SRIF, GnRH, and VP4) is not considered sufficient to enable the breadth of any selected antigen." Office Action, page 5. However, applicants respectfully disagree and submit that the claims are clearly entitled to the scope presented.

First of all, the Office's statement regarding cytokines is wholly in error. Nowhere on page 4 of the specification is reference made to the use of a cytokine as the antigenic component of the invention. In fact, applicants at page 8, lines 15-18 of the specification, expressly exclude cytokines from the purview of the invention.

With respect to the remaining allegations by the Office, it is not incumbent on applicants to detail all potential embodiments falling within the scope of the claims in order to comply with 35 USC §112, first paragraph. In fact, the CCPA in *In re Angstadt*, 190 USPQ 214, 218 (CCPA 1976), cautions against such a burdensome requirement:

> Appellants have apparently not disclosed every catalyst which will work; they have apparently not disclosed every catalyst which will not work. The question, then, is whether in an unpredictable art, section 112 requires disclosure of a test with every species covered by a claim. To require such a complete disclosure would apparently necessitate a patent application or applications with 'thousands' of examples or the disclosure of 'thousands' of catalysts. ... More importantly, such a requirement would force an inventor seeking adequate patent protection to carry out a prohibitive number of actual experiments. This would tend to discourage inventors from filing patent applications in an unpredictable area since the patent claims would have to be limited to those embodiments which are expressly disclosed. A potential infringer could readily avoid 'literal' infringement of such claims by merely finding another analogous catalyst complex which could be used in 'forming hydroperoxides'. (Emphasis in original.)

Additionally, the method by which an enabling teaching is set forth, either by the use of illustrative examples or by broad terminology, is of no importance since a

specification which teaches how to make and use the invention in terms which correspond in scope to the claims must be taken as complying with the first paragraph of §112, unless there is reason to doubt the objective truth of the statements relied upon therein for enabling support. *In re Marzocchi*, 169 USPQ 367 (CCPA 1971)

Applicants respectfully submit that the claims comply with the standards stated above. Applicants have exemplified the use of leukotoxin as a carrier molecule with three different antigens and have shown for the first time that leukotoxin is able to act as a carrier protein when fused to an antigen of interest. Applicants' work demonstrates that the claimed invention is generically operable and not simply an isolated or unreproducible success. Applicants' data, for example, demonstrate that leukotoxin polypeptides can be used as carriers for peptide hormones, such as SRIF and GnRH, as well as for unrelated viral antigens, such as bovine rotavirus VP4.

Additionally, sequences for multitudes of antigens are known and protocols for combining such sequences with leukotoxin polypeptides are explained in great detail in the specification. Also, as explained in the specification, such chimeric proteins can be readily tested for immunogenicity using routine methods, such as by comparing antibody titers against the leukotoxin/antigen fusion and antigen controls, using standard assays, such as RIAs and ELISAs, well known in the art.

Applicants submit that, given the description in the specification, the particular examples, and the level of skill in the art, a skilled artisan could readily practice the claimed invention without undue experimentation. See, e.g., *Utter v. Hiraga*, 6 USPQ2d 1709, 1714 (Fed. Cir. 1988), and *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 231 USPQ 81, 94 (Fed. Cir. 1986). The Office is reminded that even a large amount of experimentation is permitted under §112, first paragraph, provided it is routine. *Ex parte Jackson*, 217 USPQ 804, 807 (POBA 1982) (a claim is acceptable under §112 even if it requires extensive experimentation, as long as the experimentation is routine). Therefore, applicants respectfully request withdrawal of this basis for rejection.

The Action further asserts:

> Applicants' specification also only exemplifies and enables the preparation of chimeric proteins wherein the cytotoxin is the leukotoxin from *Pasteurella haemolytica*. At the time of the invention it was known that other different leukotoxins existed, i.e., from *Actinobacillus actinomycetemcomitans*...however applicants are not enabled, or were not in earlier applications, for leukotoxins other than those from *P. haemolytica*...as the term "RTX" encompasses proteins applicants had not identified at the time the invention was made. (Office Action, page 6).

Applicants disagree. Contrary to the Action's assertions, applicants describe other RTX leukotoxins including *E. coli* alpha hemolysin, and RTX toxins from *Actinobacillus pleuropneumoniae,* in the present application which has the identical specification as parent and grandparent applications Serial Nos. 08/455,970 and 07/960,932. See, e.g., page 8, line 19 through page 9, line 16 of the present application. Additionally, the great grandparent (U.S. application Serial No. 07/779,171) of the present application discusses the *E. coli* RTX toxin, alpha hemolysin, at e.g., page 17, lines 15-17. Furthermore, the Office acknowledges that at the time of the invention, other RTX leukotoxin molecules were known and it is well settled that a patent need not teach, and preferably omits, what is well known in the art. *Spectra-Physics, Inc. v. Coherent, Inc.*, 3 USPQ2d 1737 (Fed. Cir. 1987); *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 231 USPQ 81 (Fed. Cir. 1986). Accord, *Paperless Accounting, Inc. v. Bay Area Rapid Transit*, 231 USPQ 649, 653 (Fed. Cir. 1986) ("A patent applicant need not include in the specification that which is already known to and available to the public.").

Additionally, U.S. Patent No. 5,476,657, cited against the present claims, at columns 26-27, in Tables 7 and 8, shows that leukotoxins prepared from *P. haemolytica*, *Actinobacillus pleuropneumoniae* (serotypes 1 and 5) and a synthetic peptide (based on a portion of the *P. haemolytica* toxin) are cross-reactive in serological testing.

Applicants submit, therefore, that they have sufficiently enabled the use of RTX leukotoxins as claimed and should not be limited to the use of leukotoxins from *P. haemolytica*.

The Action also rejected the claims under 35 USC §112, first paragraph, alleging that the specification is only enabling for "proteins wherein the entire 'full length' sequence of the leukotoxin protein is fused to the cytokine." Office Action, page 6. The Office also asserts that "it would require undue experimentation for one skilled in the art to determine the number of epitopes on this protein and subsequently fuse them to an antigen, absent some guidance or direction for the determination of such." Office Action, pages 6-7, bridging paragraph. However, applicants disagree with these assertions.

As explained above, applicants' invention expressly excludes cytokines as antigens. Accordingly, the Office's assessment of enablement appears to be misplaced.

Furthermore, contrary to the assertion that only the use of full-length leukotoxin has been exemplified, the examples indeed describe the fusion of three representative antigens to a truncated leukotoxin polypeptide. In particular, Example 1 describes the production of a truncated a leukotoxin molecule from plasmid pAA352 (see page 35, lines 1-9) and Example 2 describes the recombinant production of chimeric molecules of the leukotoxin gene from plasmid pAA352 with sequences coding for SRIF, GnRH and VP4.

Nevertheless, applicants wish to clarify that the present claims are directed to chimeric proteins wherein the leukotoxin portion of the protein acts as a carrier molecule in order to boost the immune response to the coadministered antigen. Thus, the leukotoxin portion of the molecule does not itself act as the antigen of interest. Accordingly, the claims now recite that the leukotoxin polypeptide is "capable of activating helper T-cells." Methods of identifying regions of a molecule that stimulate helper T-cells was well within the skill of the art at the time the application was filed and

such regions would be reasonably expected to function as carriers to enhance the immunogenicity of an antigen fused thereto.

To evidence that such is the case, applicants are submitting herewith the Declaration of John G. Manns, Ph.D., provided in parent Application Serial No. 08/455,970 ("the Declaration"). Dr. Manns, a researcher in the field of reproductive immunology, attests to the fact that methods of identifying T-cell epitopes were well known and such methods could be practiced without undue experimentation. Furthermore, it is Dr. Manns' opinion that such regions could be expected to enhance the immunogenicity of an antigen fused thereto.

In particular, as explained in paragraph 4 of the Declaration, T-cell epitopes are readily identifiable based on well-defined characteristics. A T-cell epitope is generally a short peptide sequence, amphipathic in nature and comprises a hydrophobic side, for interaction with an MHC molecule, and a hydrophilic side, for interacting with the T-cell receptor. Margalit et al., *Computer Prediction of T-cell Epitopes*, New Generation Vaccines Marcel-Dekker, Inc, ed. G.C. Woodrow et al., (1990) pp. 109-116. Additionally, the amphipathic structures have an α-helical configuration (see, e.g., Spouge et al., *J. Immunol.* (1987) 138:204-212; Berkower et al., *J. Immunol.* (1986) 136:2498-2503). Hence, as explained in paragraph 4 of the Declaration, segments of leukotoxin polypeptides which include T-cell epitopes can be readily predicted using numerous computer programs. See e.g., Margalit et al., *Computer Prediction of T-cell Epitopes*, New Generation Vaccines Marcel-Dekker, Inc, ed. G.C. Woodrow et al., (1990) pp. 109-116. Such programs generally compare the amino acid sequence of a peptide to sequences known to induce a T-cell response, and search for patterns of amino acids which are believed to be required for a T-cell epitope. In fact, as explained in paragraph 5 of the Declaration, such techniques were used to identify a number of putative T-cell epitopes in amino acids 1-199 of the leukotoxin molecule. Dr. Manns states that one "would expect that a leukotoxin molecule which included one or more of these epitopes,

could be capable of enhancing the immunogenicity of an antigen fused thereto." See, paragraph 5 of the Declaration.

Evidence that such is the case comes from the data presented in paragraphs 6-11 of the Declaration. These paragraphs describe the construction and use of a leukotoxin molecule encoded by a DNA molecule having an internal deletion of approximately 1300 bp from the LKT 352 gene. This shortened leukotoxin, termed "LKT 111" has a molecular mass of approximately 52 kDa (as compared to the 99 kDa LKT 352 polypeptide). As explained in paragraph 6 of the Declaration, the molecule retains portions of the LKT 352 N-terminus containing T-cell epitopes which are necessary for sufficient T-cell immunogenicity. The DNA encoding LKT 111 was fused to DNA encoding GnRH and the resulting fusion shown to encode a chimeric protein capable of eliciting immnuneutralizing activity. Based on the knowledge in the art and the experimental evidence presented in the Declaration, Dr. Manns states in paragraph 12 of the Declaration:

> one of skill in the art could identify DNA encoding leukotoxin polypeptides capable of stimulating the production of helper T-cells, in addition to LKT 352, using techniques known in the art and without undue experimentation. Methods for identifying T-cell epitopes were known in the art and, as shown above, these methods can be used to reasonably predict portions of the leukotoxin molecule that are capable of enhancing immunogenicity of an antigen fused to the molecule.

Applicants therefore submit that the present claims indeed comply with the requirements of 35 USC §112, first paragraph and that they are indeed entitled to the scope of the present claims.

Rejections Under 35 USC §102:

Claim 37 was rejected under 35 USC §102(e) as anticipated by U.S. Patent No. 5,476,657, to Potter ("Potter 1"). The Action asserts that Potter 1 teaches the use of leukotoxin in vaccine compositions and that the leukotoxin polypeptide may be linked to

a carrier, "particularly VP6 of rotavirus." However, applicants submit that this rejection indicates a fundamental misunderstanding of the invention at issue. Particularly, the present invention utilizes leukotoxin as a carrier, not as an antigen for which specific immunity is targeted. Potter 1, on the other hand, is using leukotoxin in vaccine compositions to protect against diseases caused by *P. haemolytica*. In Potter 1, VP6 of rotavirus is a carrier protein, not the antigen of interest. Applicants submit therefore, that this basis for rejection should be withdrawn.

Additionally, claim 37 was rejected under 35 USC §102(e) as anticipated by U.S. Patent No. 5,238,823, also to Potter ("Potter 2"). The Action argues that Potter 2 "disclose the expression of a fusion protein comprising leukotoxin having substantially the sequence of leukotoxin fused to IL-2 (a selected antigen) for use as a vaccine against shipping fever pneumoniae." Office Action, page 8. However, as explained above, applicants' claims specifically exclude cytokines such as IL-2 by virtue of the definition of antigen given on page 8, lines 15-18 of the specification. Thus, Potter 2 does not anticipate the present claims and this basis for rejection should also be withdrawn.

Rejections Under 35 USC §103:

Claim 37 was rejected under 35 USC §103, as being unpatentable over any one of Lorberboum-Galeski et al., *Proc. Nat. Acad. Sci. USA* (1988) 85:1922-1926 ("Lorberboum-Galeski"), Williams et al., *Protein Engineering* (1987) 1:493-498 ("Williams"), U.S. Patent No. 4,675,382 to Murphy ("Murphy"), or U.S. Patent Nos. 4,935,233 to Bell et al. ("Bell 1") and 5,114,711 to Bell et al. ("Bell 2"), in view of Highlander et al., *DNA* (1989) 8:15-28 ("Highlander"), Strathdee et al., *J. Bacteriol.* (1989) 171:916-928 ("Strathdee") or Lo et al., *Infect. Immun.* (1985) 50:667-671 ("Lo") and further in view of U.S. Patent No. 5,028,423 to Prickett ("Prickett"). The Examiner contends that each of the primary references, Lorberboum-Galeski, Williams, Murphy, Bell 1 and Bell 2, discloses the recombinant production of a protein comprising a

cytokine and various different cytotoxins. The secondary references are said to describe the leukotoxin gene from *P. haemolytica* and the tertiary reference, Prickett, is alleged to disclose immunogenic conjugates comprising small peptide regions of leukotoxins. Applicants respectfully traverse this rejection.

In particular, applicants submit that the Action has failed to establish a *prima facie* showing of obviousness, as no conceivable combination of the cited references suggests all of the features of the claimed invention. Accordingly, the instant rejection is improper and therefore traversed.

In order to establish a *prima facie* case of obviousness, the references must be considered in their entirety for what they fairly teach to one of ordinary skill in the art. *In re Hedges*, 228 USPQ 685 (Fed. Cir. 1986). It is the claimed subject matter *as a whole* which must be considered under §103 to determine obviousness. See, e.g., *Panduit Corp. v. Dennison Mfg. Co.*, 1 USPQ2d 1593 (Fed. Cir. 1987); *Stewart-Warner Corp. v. City of Pontiac*, 266 USPQ 676 (Fed. Cir. 1985). Thus, changes from the prior art must be evaluated in terms of the whole invention, including whether the prior art provides any teaching or suggestion to one of ordinary skill in the art to make the changes that would produce applicant's claimed invention. *Northern Telecom, Inc. v. Datapoint Corp.*, 15 USPQ2d 1321 (Fed. Cir. 1990).

In this regard, each of the cited primary references pertains to attachment of a cytokine to a cytotoxin. The purpose of creating the composite molecules of the primary references was to increase the cytotoxicity of the toxic component. Applicants, on the other hand, wish to elicit an enhanced immune response toward a selected antigen in order to provide immunity in immunized subjects towards diseases caused by the pathogen from which the antigen is derived. The chimeric proteins of the invention comprise an RTX leukotoxin polypeptide coupled to an antigen of interest. Cytokines are explicitly excluded from the definition of antigen at page 8, lines 15-18. The leukotoxin portion of the chimeric molecule of the present invention serves as an immune response

potentiator, not as a toxic component, by providing T-cell epitopes for activating helper T-cells in order to enhance immunity to the selected antigen.

This is in sharp contrast to the fusion proteins and chemical conjugates cited against the claims by the present Action, wherein those references teach how to kill cells with cytotoxic proteins. There is absolutely no hint in any of the primary references that the systems could be used to enhance the immunogenicity of a selected antigen. Accordingly, this art is not analogous to applicants' chimeric proteins.

With respect to the secondary references, applicants note that those references pertain merely to cloning *P. haemolytica* leukotoxin. Again, none of the references suggest combining the resultant leukotoxin with an antigen of interest that is not a cytokine, in order to enhance an immune response.

Prickett (the tertiary reference) does not provide the missing link. Prickett pertains to chemical conjugates of small peptide fragments of leukotoxin to conventional carrier proteins, such as albumins and globulin fractions. (See column 3, lines 24-33). The leukotoxin is clearly not acting as an immunological carrier in this context. Accordingly, applicants submit that, when the cited references are considered in their entirety for what they fairly teach to one of ordinary skill in the art, none of the references, alone or in any combination, provides the requisite suggestion for a chimeric protein comprising a leukotoxin molecule coupled to a selected antigen. The Examiner is respectfully reminded that something which was unknown cannot have been obvious. *In re Newell*, 13 USPQ2d 1248, 1250 (Fed. Cir. 1989).

Finally, the fact that the Office relies on nine references in making the present rejection is, in and of itself, probative of nonobviousness. One can only surmise that the Office, in making the present rejection, has relied on hindsight reconstruction, an impermissible standard. As stated by the Court of Appeals for the Federal Circuit, "[i]t is impermissible to use the claimed invention as an instruction manual or 'template' to piece together the teachings of the prior art so that the claimed invention is rendered obvious."

*In re Fritch*, 23 USPQ2d 1780, 1784 (Fed. Cir. 1992). Thus, it is insufficient merely to show that some or all of the elements of the invention are present in the prior art and possess characteristics of the elements of the instant invention.

Since none of the references, either alone or in combination, teaches or suggests the use of an RTX leukotoxin polypeptide coupled to an antigen of interest that is not a cytokine, in order to enhance immunity thereto, applicants submit that the Action has failed to establish a *prima facie* showing of obviousness. Reconsideration and withdrawal of the rejection of claim 37 under 35 USC §103 is therefore respectfully requested.

## Conclusion

Applicants respectfully submit that the claims comply with the requirements of 35 USC §112, and define an invention which is novel and nonobvious over the art. Accordingly, allowance is believed to be in order, and an early notification to that effect would be appreciated.

If the Examiner notes any further matters which she believes may be expedited by a telephone interview, she is requested to contact the undersigned attorney at (650) 325-7812.

Respectfully submitted,

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Currently Elected Claims (08,976,566)

37. (Amended) A chimeric protein comprising a leukotoxin polypeptide capable of activating helper T-cells, coupled to a selected antigen, wherein said leukotoxin polypeptide is an RTX leukotoxin from a bacterium selected from the group consisting of *Pasteurella haemolytica*, *E. coli* and *Actinobacillus pleuropneumoniae*.

40. The chimeric protein of claim 37, wherein said leukotoxin polypeptide is coupled to gonadotropin releasing hormone (GnRH), or an epitope thereof.

41. The chimeric protein of claim 40, comprising the amino acid sequence of SEQ ID NO:12.

44. (New) The chimeric protein of claim 37, wherein the leukotoxin polypeptide is a *Pasteurella haemolytica* leukotoxin polypeptide.

45. (New) The chimeric protein of claim 40, wherein the leukotoxin polypeptide is a *Pasteurella haemolytica* leukotoxin polypeptide.